

## Policy & Procedure (P&P)

Policy Title :

### Du Testing

Department	Index No.	Scope
Laboratory & Blood Bank	LAB-065	All Blood Bank and Nurses
Issue Date	Revision NO	Effective Date
1432/06/06	3	1440/07/23
Review Due Date	Related Standard NO.	Page Number#
1442/07/23	CBAHI ( LB.50.1.3 )	9

#### 01. Policy:

The Du test will detect a weak expression of the D antigen by incubating the patient cells with anti-D serum at 37°C followed by the indirect antiglobulin test.

01.1. There is instance when an accurate Rh type cannot be determined through routine testing

01.1.1. If the new-born's cell is coated with maternal IgG anti-D in utero, very few D Ag sites will be available to react with reagent anti-D.

01.1.2. Warm autoimmune hemolytic anemia, Abs are directed against the patient's own red cell and react as though they are Rh specific.

01.2. Three different mechanisms have been described that can explain the weakened expression of the D Ag

01.2.1. Genetic weak D: Inheritance of D genes that code for a weakened expression of the D Ag.

01.2.2. C Trans: (a position effect or gene interaction effect). The Rh Ag on the red cell is normal, but the steric arrangement of the C Ag in relationship to the D Ag appears to interfere with expression of the D Ag. The D Ag expressed appear to be complete but few in number.

01.2.3. D Mosaic or partial D: One or more parts( sub-units )of the D Ag are missing( the complete D Ag had four parts designated Rh<sup>A</sup>, Rh<sup>B</sup>, Rh<sup>C</sup>, Rh<sup>D</sup>).

#### 02. Definition :

N/A



### 03. Purpose :

- 03.1. Some red cells express the D antigen so weakly that most anti-D reagents do not directly agglutinate the cells. Weak D expression can be demonstrated most reliably by the indirect antiglobulin test after incubating the cells with anti-D serum.

### 04. Procedure :

Weak D or Du can be detected by one of the following methods:

#### 1. Tube Method

##### 1. Specimen Requirements

Blood collected with or without anticoagulant may be used. Specimens collected in EDTA. Testing should be performed as soon as possible. If a delay in testing occurs, specimens should be stored at 2-8°C and tested within 24 hours. No patient preparation is required.

##### 2. Materials

- 2.1. Glass test tubes
- 2.2. Disposable Blood Bank pipettes
- 2.3. Isotonic saline 0.85% NaCl
- 2.4. Calibrated serologic centrifuge
- 2.5. 37° C water bath
- 2.6. Timer
- 2.7. Microscope and slides
- 2.8. Cell washer

##### 3. Reagents

- 3.1. Anti-D.
- 3.2. Rh control or 22% Bovine albumin.
- 3.3. Anti-human serum for the anti-globulin test.
- 3.4. Coombs control cells.

##### 4. Method

**Note:** The initial anti-D testing was performed by the tube technique, the same tubes and anti-D, Rh control may be used directly for the Du testing. In this case, proceed directly to step 6 of the procedure.



- 4.1. Wash an appropriate volume of patient red blood cells two times with isotonic saline.
- 4.2. Prepare a 3% to 5% suspension of red blood cells in isotonic saline.
- 4.3. Label two test tubes with the patient identification and the Rh control.
- 4.4. Add one drop respectively of anti-D and Rh control to the appropriately labeled tube.
- 4.5. Using a plastic pipette, add one drop of the cell suspension to each test tube. Mix well.
- 4.6. Incubate the two tubes at 37°C for 15-30 minutes.
- 4.7. After the incubation, wash the cells three times with the isotonic saline.
- 4.8. To each tube, add two drops of anti-human serum.
- 4.9. Mix and centrifuge according to the optimum AHG time for the centrifuge.
- 4.10. Resuspend the cells by gentle agitation and examine macroscopically for agglutination. If negative examine microscopically for agglutination.
- 4.11. If the test is negative, add known IgG sensitized cells (Coombs control cells). Centrifuge and observe for agglutination. These cells must agglutinate for a negative test to be valid.
- 4.12. Record results immediately in blood grouping register.

#### 5. Quality Control

Reagents are to be tested with appropriate positive and negative controls set daily and the results are recorded in a special log sheet. See "Daily Quality Control" for procedure (LAB-116).

#### 6. Reporting Results

##### 6.1. Interpretation

- 6.1.1. When agglutination appears in the tube with anti-D serum and not in the control tube, report the patient's Rh as D positive. It is incorrect to report a patient's type as "Rh negative Du positive". Du positive patients should receive Rh negative blood. Du positive donors' blood should be labeled as Rh positive.
- 6.1.2. If there is no agglutination with anti-D or Rh control, the patient is classified as Rh negative. All Rh-negative results must be recorded in the patient file.
- 6.1.3. Red cells which agglutinate with both anti-D and Rh control cannot be accurately tested to the Du antigen. In this situation, a potential recipient should be given Rh negative blood; if the cells are from a donor they should not be used for transfusion.



except after full investigations are completed. Reaction will show the following patterns.

Anti-D	Rh Control	Interpretation
-	-	<b>Rh negative</b>
+	-	<b>D positive</b>
+	+	<b>Test invalid</b>

#### 6.2. Reporting Results

#### 7. Limitations

- 7.1. Mixed field agglutination in the Du test on a woman recently delivered may indicate a mixture of maternal Rh negative and fetal Rh-positive blood.
- 7.2. Red cells demonstrations a positive direct antiglobulin test cannot be accurately tested for Du.
- 7.3. Invalid positive test results may be obtained from the blood tested is from a person with autoantibodies or abnormal serum proteins.
- 7.4. Inadequate washing of red blood cells will allow trace amounts of residual unbound globulin to neutralize the antiglobulin serum causing a false negative result. Adding coombs check cells which then agglutinate assures that this had not happened.
- 7.5. Over-centrifugation and under-centrifugation must be avoided.
- 7.6. False positive results
  - 7.6.1. If the cells and ant-sera remain together too long before the test is read, the protein medium may produce rouleaux which resemble agglutination.
  - 7.6.2. Antisera contaminated with bacteria and foreign substances.
- 7.7. False negative results
  - 7.7.1. Too heavy cell suspension in the tube test may result in poor agglutination.
  - 7.7.2. Cells processing weak antigens (D) may not show agglutination.

## 2. Crossmatching using column technology and the Gel Micro typing System

### 2.1. Principle

A specific red cell solution is added to the gel contained in a special micro tube. The gel acts as a trap, the free red blood cells pellet in the bottom of the tube while agglutinates are trapped(fixed)in the top of the gel for hours.





## وزارة الصحة

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### 2.2. Equipment/Materials

- 2.2.1. ID Centrifuge
- 2.2.2. ID Incubator 37°C
- 2.2.3. ID working table
- 2.2.4. ID pipettor EP-3/FP/FP-2/FP-3
- 2.2.5. ID Dispensary
- 2.2.6. ID Suspension tubes
- 2.2.7. ID Disposable tips

### 2.3. Reagents

- 2.3.1. ID Diluent 2 (modified LISS)
- 2.3.2. Rh control or 22% Bovine albumin

### 2.4. Micro typing cards

LISS/Coombs (Profile: -6 Coombs tests)

### 2.5. Sample

- 2.5.1. Red cell concentrate/Whole blood from donor.

### 2.6. Test procedure

- 2.6.1. Allow all reagents to reach room temperature before use.
- 2.6.2. Identify the ID-micro typing card with the donor's name and number and another one for Rh control, remove the aluminum foil.
- 2.6.3. Prepare a 0.8% suspension of donor unit red cells as follow:
  - 2.6.3.1. 10-micron reed cell concentrate + 1 ml ID-diluents 2; or
  - 2.6.3.2. 20-micron whole blood + 1 ml ID-diluents 2 mix well.
- 2.6.4. Add 50 U of donor red cell suspension to the appropriate micro tube.
- 2.6.5. Add 25U of Anti-D & Rh control to the appropriate micro tube.
- 2.6.6. Incubate the micro typing card for 15 minutes at 37c in the ID-Incubator.
- 2.6.7. Centrifuge the micro typing card for 10 minutes in the ID-centrifuge.
- 2.6.8. Interpret the result.

### 2.7. Interpretation

- 2.7.1. Positive reaction, grade 1-4, in the micro tube and negative for control indicates Weak D.
- 2.7.2. Negative reaction in both micro tubes indicates that the donor is Rh negative.



### 2.8. Quality Control

Reagents & microtubes are to be tested with appropriate positive and negative controls daily (ID-Internal Quality Control) and the results recorded. See "Daily Quality Control" for procedure.

### 3. Cross matching using TANGO optima System (Fully automated machine)

#### 3.1. Reagents

<b>Solid screen™ II Strip</b>	Plates with 12 strips per plate. The strips are coated with protein A. The plates can be used for weak D testing.
<b>MLB 2</b>	Modified LISS 2 (Low Ionic Strength Solution) is an antibody enhancement media specifically formulated for use with the Solid screen™ II test system. The MLB 2 is used in the preparation of a 1% cell suspension of sample red blood cells. The reagent is supplied in 50 mL vials.
<b>AL severs Solution</b>	Used as a suspension medium for red blood cells.
<b>Anti-Human Globulin Anti-IgG Solid screen™ II</b>	Anti-Human Globulin is anti-IgG obtained from immunized rabbits. The Anti-Human Globulin is used to demonstrate the presence of antibodies bound to the reagent red blood cell that do not cause direct agglutination. The reagent is colored green and supplied in 55 mL vials.
<b>Solid screen™ II Anti-D (RH1) Blend</b>	For the detection of weak D and partial D antigens. It is used to test samples which have been tested negative with IgM Anti-D using Retype™ S.

#### 3.2. Materials

3.2.1. Glass test tubes

3.2.2. N rack of the Tango Optima machine



### 3.2.3. Calibrated serologic centrifuge

### 3.3. Sample

Well centrifuged EDTA whole blood

### 3.4. Principle of the test

Solid screen™ II Strip plates are composed of test strips (12 strips with 8 wells each) that have been coated with Protein A.

Protein A has a high binding affinity for the Fc region of immunoglobulins

The method used for the Cross-matching test on the Solid screen™ II Strip is solid phase adherence. If red blood cell alloantibodies and/or autoantibodies are present in the sample to be tested they will bind to the donors red blood cells.

Unbound antibody is removed by washing.

After adding Anti-Human- Globulin specially formulated for the Solid screen™ II assay and centrifugation Anti-Human-Globulin forms a link between the protein A on the surface of the microwell and the donors red blood cells. Thereby a layer of red blood cells will form on the surface of the microwell (compatible).

But if no red blood cell alloantibodies or autoantibodies are present in the sample, the cells will not get coated with antibodies and therefore will not form a cell layer on the surface of the microwell but sediment to the bottom of the well to form a cell button.

### 3.5. Test procedure

3.5.1. Centrifuge Patients & donor blood sample & put them in (N) Rack.

3.5.2. Open the sample door & put the (N) Rack in its correct place.

3.5.3. Push the Rack button to select the desired rack on the screen.

3.5.4. Press manual entry button & write the ID number of patients & donors' sample.

3.5.5. Press accepted entry button.

3.5.6. Press the button to select the test & close the sample door.

3.5.7. Press start button to start the test.

3.5.8. After the end of the test validate the results.

3.5.9. Print out the results.

### 3.6. Quality Control

Control Set QC contain test erythrocytes with defined antigen pattern for ABO, Rh (D), phenotyping {Rh



(CEce) and Kell} as well as isoagglutinin's for reverse typing.

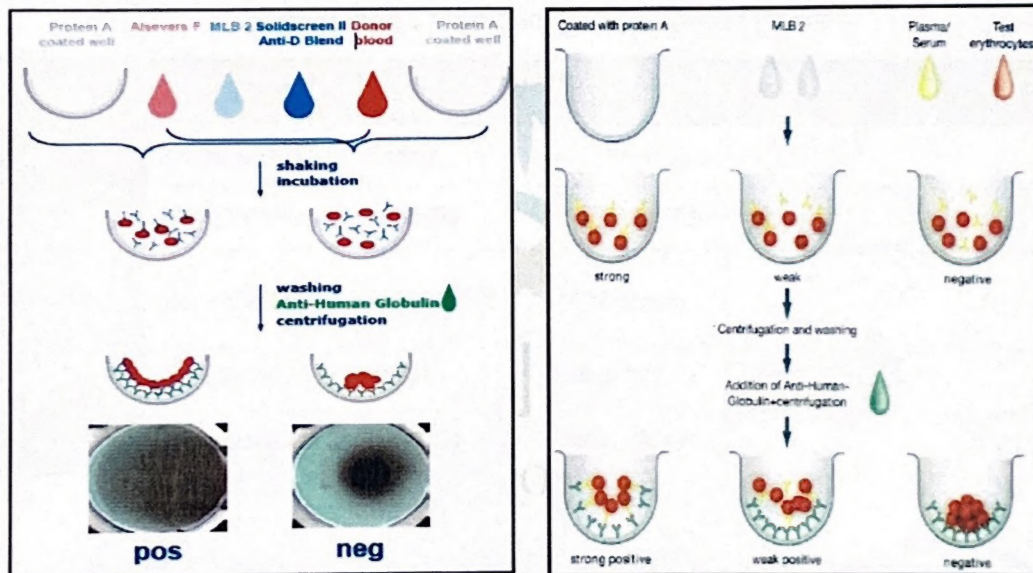
3.6.1. Group O Neg

3.6.2. Group AB Pos

3.6.3. Group A Neg

3.6.4. Group O Pos

3.6.5. Solid screen 11-control is used for Antibody screen QC.



## 05. Responsibilities :

05.1. All Blood Bank staff of Al-Qunfudah General Hospital.

## 06. Equipment & Forms

06.1. Blood Grouping Book

## 07. Attachment :

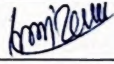
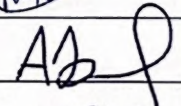

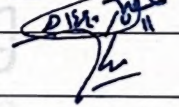

N.A



#### 08. Reference

- 08.1. King Abdul Aziz Hospital.
- 08.2. Tango Optimo manual book.
- 08.3. Manual for Blood Bank in Arab countries.

#### Preparation , Reviewing & Approval Box

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